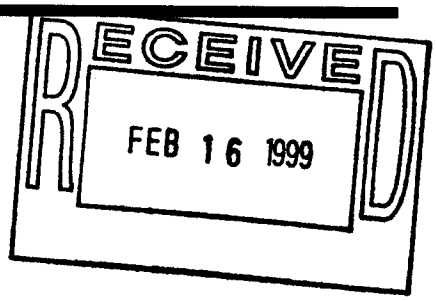
The Sapphire Group™



12 February 1999

VIA FAX (919-541-0947); ORIGINAL TO FOLLOW

Dr. C. Wm. Jameson
The U.S. National Toxicology Program
79 Alexander Drive, Building 4401, Room 3127
PO Box 12233, MD WC-05
Research Triangle Park, NC 27709

Dear Dr. Jameson:

I have been commissioned by the Oxygenated Fuels Association (OFA) to write on its behalf and offer supplemental comments on the question of whether to list methyl-*t*-butyl ether (MTBE) as a "reasonably anticipated human carcinogen" in NTP's forthcoming Biannual Report on Carcinogens. The comments are submitted in response to NTP's request for public comments issued in the Federal Register (Vol. 63, No. 239, pp 68783-68785).

OFA supports the conclusion to not list MTBE made by both NTP's inter-agency review group and NTP's external review group. OFA is confident that all the data addressing the matter of carcinogenicity are insufficient to meet NTP's criteria for listing, and that, therefore, MTBE should not be listed in Ninth Edition of NTP's report. Of particular note is that the International Agency for Research on Cancer (IARC), shortly before NTP's external reviewers considered MTBE, concluded that MTBE is "not classifiable as to its carcinogenicity for humans." Shortly after the IARC concluded its review, an external review committee of California's Office of Environmental and Human Hazard Assessment (OEHHA) also concluded that all relevant data on MTBE were insufficient to list MTBE as a "carcinogen known to the State of California."

The following are our major reasons in support of not listing MTBE under any designation of carcinogenicity in the Ninth Edition of NTP's list of carcinogens. The comments supplement OFA's written comments dated 20 March 1998.

- No data or justification exists to list MTBE in NTP's Report as a "known to be carcinogen."

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- With regard to possible carcinogenicity in non-human species:
  - (1) MTBE is not a genotoxicant and is highly unlikely to be a genotoxic carcinogen because it has been shown repeatedly in numerous genotoxicity tests to cause no genetic alterations. In the one test that was positive, research findings indicate that it may well be due to methodological aberration. The enclosed report by a leading expert in genetic toxicology substantiates the conclusion that MTBE is not genotoxic and explains the lack of relevance of the only published positive finding.

  - Compelling evidence exists to indicate that the male rat kidney tumors are likely to result from secondary, non-genotoxic tissue damage caused by otherwise highly toxic doses of MTBE—doses unlikely to be experienced by humans; and this mechanism is unlikely to exist in humans. The pattern of kidney tumors in male rats and the results of extensive mechanistic studies clearly indicate that this tumor type is specific to the male rat exposed to MTBE at toxic doses, and that this tumor type is not relevant to humans exposed to MTBE. Of particular note, the NRC committee stated that

    > "the male rat kidney-tumor data probably should not be used for (estimating the cancer potency of MTBE) in light of the new information on its probable causation, *i.e.* α_{2u}-globulin nephropathy, which is thought to be unique to the male rat and **not relevant to humans** (emphasis added)."

  - Mounting evidence strongly suggests that the female mouse liver tumors are the result of high, non-genotoxic doses of MTBE interfering with the tumor suppressor mechanisms of estrogen in female mouse liver—mechanisms that are not present in human liver. The pattern of liver tumors in the female mouse and the results of mechanistic studies suggest that this tumor type is specific to the female mouse exposed to MTBE at toxic doses, and that this tumor type is not relevant to humans exposed to MTBE.

  - Evidence suggests that the testicular tumors in rats were likely to result from factors other that MTBE.

  - The findings of the gavage study are considered sufficiently flawed and provide no evidence for judging whether MTBE might be reasonably anticipated to be a human carcinogen. Therefore, this study should not be used

in judging whether MTBE should be listed by NTP in the next edition of its Report on Carcinogens.

In conclusion, MTBE has been tested extensively to determine whether it can cause cancer and, if so, whether the findings are relevant for humans exposed to ambient levels of this compound. Although toxic doses of MTBE have caused cancer in laboratory rats and mice, the preponderance of evidence indicates that it is reasonably anticipated to not be a human carcinogen. These five conclusions are widely supported by several in-depth evaluations by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1997), the U.S. Health Effects Institute (HEI, 1996), and the National Academy of Sciences (NRC, 1996). Therefore, the overall data strongly support the conclusion that MTBE is not reasonably anticipated to be a human carcinogen.

Thank you for considering these comments in NTP's ensuing deliberations.

Sincerely,

Robert G. Tardiff, Ph.D., ATS
President and CEO

cc: J. Kneiss (Oxygenated Fuels Association)

Attachment

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Evaluation of the Genotoxicity of Methyl-tertiary-butyl-ether (MtBE)

Prepared by
David Brusick, Ph.D., ATS

Prepared for
The Sapphire Group, Inc.
Bethesda, MD

On Behalf of the Oxygenated Fuels Association
Arlington, VA

January 1999

Background

Methyl-tertiary-butyl-ether (MtBE) has been evaluated for possible genetic toxicity in a wide range of *in vitro* and *in vivo* test methods. The results of these studies have been summarized in a number of publications and expert panel reviews (Duffy et al., 1992; Gilbert and Calabrese, 1992; Dutch Expert Committee on Occupational Standards, 1994; NSTC, 1996; ECETOC, 1997; Stern and Tardiff, 1997). Although one set of *in vitro* data from the Mouse Lymphoma assay was positive, the consensus across the evaluations is that MtBE is not genotoxic. Several hypotheses have been proposed to explain the positive Mouse Lymphoma data (Gilbert and Calabrese, 1992), attributing the mutagenic effects to a unique susceptibility of the method to toxic metabolic products (aldehydes, acids and alcohols).

Objectives

I have reviewed all of the study data and examined relevant sections of the summaries cited above. Additional data has been published since these reviews (Kado et al, 1998) which adds support to the presumption that MtBE is not a mutagen or clastogen.

I will summarize the data with special attention to the Mouse Lymphoma data and some of the other *in vitro* studies which appeared to be equivocal.

Evaluation

Tables 1 and 2 summarize the results of the *in vitro* and *in vivo* genetic toxicology studies which have been performed on MTBE. The two tables are modified versions of table published in the ECETOC report. They include new data published since the 1997 ECETOC report and reflect my interpretation of some of the reported results in the original versions of the Tables.

In Vitro Results

The only addition to Table 1 are the results from the Ames test published by Kado et al (1998). While the Kado et al. results are similar to the results of all prior Ames tests, they are important for two reasons. First, the studies were done in suspension cultures to minimize compound loss and replicate the types of exposure conditions used in the Mouse Lymphoma assay, and second, strain TA104 was employed as a check for aldehyde mutagens (this tester strain is known to respond to genotoxic aldehydes such as formaldehyde). Several different concentrations of S9 were employed by Kado et al. in order to facilitate production of metabolites. Negative responses in this study provide stronger support for the presumption that neither MtBE or metabolites are mutagenic.

The original table, as presented in the ECETOC report gave equivocal responses (+/-) for the Mouse Lymphoma study (+S9), for SCE induction in CHO cells (+S9) and for mutation induction in V79 cells.

After reviewing all of the data from these studies, my opinion is that the SCE and V79 mutations studies are negative. There were two concentrations in the first of two trials with S9 mix which showed a slight increase in SCEs. The increases were absent from an independent repeat study, and the internal variability of SCE frequencies across dose levels of this study was great enough to account for these two non-reproducible increases. The data reported for the V79 mutation evaluation was completely negative and I could not find any evidence in the data supporting an equivocal (+/-) classification. None of the mutation frequencies were out of the normal range for this assay.

In my opinion the responses obtained in the Mouse Lymphoma assay for MtBE (+S9) were not equivocal. The increases were completely reproducible in a total of 5 trials (including both a commercial sample of MtBE and a 99.99% pure sample). A common feature of the *in vitro* mammalian cell assays was that the addition of S9 mix significantly reduced the amount of MtBE that could be added to the target cells suggesting that a intermediate was responsible for any bioactivity. The increased mutation frequencies were obtained at toxicity levels within the acceptable range for this assay and were generally dose related. Consequently, the results of this assay should be classified as positive. My concern is that the results with S9 mix are not related to MtBE directly, but are the result of one or more of its metabolites. The ARCO report (1980) suggests that formaldehyde is produced from MtBE and may be the mutagen detected in the Mouse Lymphoma because other studies have demonstrated that formaldehyde is detected in this assay. Follow-up studies trying to establish the validity of this hypothesis were less than convincing; however, data from metabolism studies in rats with MtBE indicate that another intermediate is formic acid. Both acids and aldehydes are prone to give positive responses in the Mouse Lymphoma assay, particularly when S9 is present. Considering the fact that none of the other *in vitro* assays for gene mutation gave any evidence of increased mutation, the results from the Mouse Lymphoma assay must be considered a unique response of these cells to one or more of the toxic metabolites and unlikely to be generalized to other assays.

In Vivo Results

The addition to Table 2 was a mouse micronucleus assay conducted by Kado et al. (1998). MtBE in this study was administered orally up to 1.75 gm/kg without producing clastogenicity. The entire set of *in vivo* studies did not provided any indication that MtBE is mutagenic, clastogenic or in any way alters DNA integrity in rodent models. The general consensus related to interpretation of genetic toxicology data is that *in vivo* studies have more relevance in hazard assessment and should be used to place *in vitro* data in perspective.

The *in vivo* studies conducted with MtBE were performed using oral and inhalation exposure roots. Both are relevant to potential exposures. The dose levels were significantly higher than any anticipated human exposures and provide a substantial margin of safety for any possible DNA damage.

Tertiary-butyl Alcohol (TBA)

In vivo TBA is one of the major metabolites of MtBE administration. The National Toxicology Program (NTP) has subjected TBA to a series of *in vitro* and *in vivo* genetic toxicology studies (Zeiger et al., 1987; McGregor et al., 1988; NTP, 1995). The tests included in this series were:

- Ames test
- Mouse Lymphoma assay
- Cytogenetic analysis in CHO cells *in vitro*
- SCE analysis in CHO cells *in vitro*
- Mouse Micronucleus assay *in vivo*

The conclusions reach following evaluation of these studies were that none of the tests produced clear evidence that TBA induced genotoxic effects. The results of these studies, therefore, are consistent with the results from MtBE studies and support the presumption that MtBE does not pose a genotoxic hazard.

Other Information

Rosenkranz and Klopman (1991) published an analysis of the probability that MtBE would be genotoxic based on its structure. The SAR system they have developed has been reasonably accurate in detecting mutagenic structures and their results indicate that MtBE does not possess the structural attributed associated with genotoxicity.

General Summary

There is no universally accepted set of criteria to classify agents as non-genotoxic. Most regulatory bodies and other advisory groups who must evaluate complex data sets use a weight-of-evidence method which accepts a minimum number of discordant responses and will classify agents as non-genotoxic with occasional positive responses in large data sets, especially if the positive effects are *in vitro*.

The genetic toxicity database for MtBE is extensive and, in large part, unambiguous. Among 15 studies measuring all aspects of genetic toxicity (mutation, clastogenicity, recombination and DNA repair), there was only one study which produced positive results. In my opinion this response was induced by formic acid or formaldehyde and is unique to the Mouse Lymphoma L5178Y cell line (Cifone et al., 1987). In view of the full data set, MtBE should be classified as non-genotoxic.

David Brusick, Ph.D., ATS

1/4/99
Date

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Table 1
Genotoxicity of MTBE *in vitro*

Test system	End point	Concentration	Results -S9	Results +S9	Reference*
Salmonella typhimurium (TA 1535, 1537, 1538, 98, 100	Reverse mutation	0.007-7.40 mg/plate (Two samples)	-	-	ARCO, 1980; Litton Bionetics Inc., 1978
Salmonella typhimurium (TA1535, 1537, 1538, 98, 100)	Reverse mutation	0.341-5.41 mg/plate	-	-	Seeberg and Cinelli, 1989.
Salmonella typhimurium (TA1535, 1537, 1538, 98, 100)	Reverse mutation	0.004-2.66 mg/plate	-	-	Hüls AG, 1991
Salmonella typhimurium (TA98, 100, 104, 1535)	Reverse mutation	30-7400 mg/table	-	-	Kado et al 1998
Saccharomyces cerevisiae	Gene conversion	0.007-7.40 mg/plate	-	-	ARCO, 1980
Mouse lymphoma cells L5178Y TK +/-	Forward mutation	0.229-7.40 mg/ml	-	ND	ARCO, 1980; Litton
		0.118-4.44 mg/ml (Two samples)	ND	+	Bionetics Inc., 1979a
Chinese Hamster Ovary cells	SCE; chromosome aberrations	0.003-0.740 mg/ml	-	ND	ARCO, 1980; Litton
		0.009-3.70 mg/ml	-	-	Bionetics Inc., 1980
Chinese Hamster v69 cells	Forward mutation	0.003-5.40 mg/ml	-	ND	Seeberg, 1989a
		0.148-1.33 mg/ml	ND	-	
Rat hepatocytes	Unscheduled DNA synthesis	0.148-5.40 mg/plate	-	ND	Seeberg, 1989b

-S9 or +S9 = assayed in the absence or presence of S9 activation
- = no mutagenic activity
+/- = equivocal mutagenic activity (see text)
ND = test not performed

* Full citations for all studies listed in Tables 1 and 2 (with the exception of Kado et al 1998) are given in ECETOC Technical Report No. 72. Methyl *tert*-Butyl Ether (MTBE) Health Risk Characterisation. CAS No. 1634-04-4 (EINECS No. 216.653.1) June 1997.

Kado et al 1998 is cited in Mutation Research 412 (1998) pp. 131-138. Genotoxicity testing of methyl tertiary-butyl ether (MTBE) in the Salmonella microsuspension assay and mouse bone marrow micronucleus test.

Table 2
Genotoxicity of MTBE *in vivo*

Species	End point	Route	Dose	Result	Reference
Rat (male Sprague-Dawley)	Chromosome aberrations (tibial bone marrow)	oral (gavage)	30, 96, and 296 mg/kg (as a single dose or five consecutive daily doses)	No clastogenic activity	ARCO Chemical Company, 1980 Litton Bionetics Inc., 1979b
Rat (male and female, F344)	Chromosome aberrations (femoral bone marrow)	inhalation	0, 2,880, 14,400, 28,800 mg/m^3	No clastogenic activity	Vergnes and Morabit 1989
Mouse (male and female, CD-1)	Micronucleus (femoral bone marrow)	inhalation	0, 1440, 10,800, 28,800 mg/m^3	No clastogenic activity	Vergnes and Kintigh, 1993
Mouse (male and female, CD-1)	Micronucleus	oral	0, 0.25, 0.5, 1.0,1.5, 1.75 gm/kg	No clastogenic activity	Kado et al. 1998
Mouse (male and female, CD-1)	Unscheduled DNA synthesis in hepatocytes (*in vivo/in vitro*)	inhalation	0,1440, 10,800, 28,800 mg/m^3, (6h/d on 2 consecutive days)	No increase in UDS	Vergnes and Chun, 1994
Drosophila melanogaster	Sex-linked dominant lethal	ingestion	0.3% in sucrose	No increase in recessive lethal events	Hazleton Laboratories America Inc., 1989

COVANCE
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David J. Brusick, Ph.D., A.T.S.

Dr. Brusick is currently Vice President of Mammalian Toxicology at Covance Laboratories, North America. Dr. Brusick was awarded his doctoral degree in genetics by Illinois State University in 1970, and was awarded a postdoctoral research position as a National Academy of Sciences research associate at the Food and Drug Administration's Genetic Toxicology Branch. Dr. Brusick is past president of the U.S. Environmental Mutagen Society (1978-79) and is adjunct associate professor of microbiology and genetics at Howard University Medical School and George Washington University, respectively. He is the author of over 100 scientific publications, including a textbook, *Principles of Genetic Toxicology* (Second Edition, 1987); was the editor of *In Vitro* Toxicology (1988-1993), a journal of cellular and molecular toxicology; and edited a volume entitled *Method for Genetic Risk Assessment*. Dr. Brusick has served as a member of numerous NAS committees and chaired an NAS/NRC subcommittee on the role of DNA adducts in toxicology testing. He is chairman of the International Commission for the Protection Against Environmental Mutagens and Carcinogens and a member of the Technology Transfer Committee for the Center for Alternative to Animal Testing at John Hopkins University. Dr. Brusick is a fellow of the Academy of Toxicological Sciences. His interests include basic and applied research in mutagenic and carcinogenic mechanisms and the application of biotechnology techniques to safety testing method development.